

## *Instructional lecture*

# **Tissue engineering of cartilage: the road a group of researchers have traveled**

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### **Introduction**

Articular cartilage is composed of chondrocytes and matrix, and it is difficult for cartilage to heal after injury. In 1743, Hunter had stated that, “cartilage once destroyed is never recovered.”<sup>1</sup> This was true until 2003, when Koshino and colleagues reported regeneration of degenerated articular cartilage after high tibial valgus osteotomy for medial compartmental osteoarthritis of the knee.<sup>2</sup> In their report, 47 (32.2%) of 146 knees were totally covered by new regenerated fibrocartilage or hyaline cartilage. Histology also demonstrated hyaline cartilage regeneration on previously degenerated medial femoral condyle. However, the regeneration occurred in only one-third of the knees. Many other treatments have also been used directly on focal lesions of the articular cartilage of the knee, including abrasion arthroplasty,<sup>3</sup> microfracture,<sup>4</sup> autogenous chondrocyte implantation,<sup>5</sup> allogeneous osteochondral transplantation,<sup>6</sup> and autogenous osteochondral transplantation (“mosaicplasty”).<sup>7</sup>

Johson<sup>3</sup> reported a 77% success rate at a 2-year follow-up for abrasion arthroplasty, although the 5-year follow-up success rate decreased to 51%–53%. The disadvantage of this technique is that abrasion may produce thermal necrosis. In contrast, Steadman et al.<sup>4</sup> used a microfracture technique that increased the stiffness of the bone and produced no heat necrosis, thus preserving the subchondral bone plate. After penetration of the bone plate, continuous passive motion was used for 6–8 weeks with limited weight bearing. This yielded a 75% success rate after 7 years of follow-up.

Autogenous chondrocyte implantation (ACI) was reported in 1994. At a 2-year follow-up, 87% good to excellent results were reported in femoral lesions,<sup>8</sup> but patellar lesions tended to have a worse outcome. Peterson et al.<sup>9</sup> reported that at 2–9 years of follow-up good results were obtained in 92% of isolated femoral condyle lesions, 65% of patellar lesions, and 67% of multifocal chondral damage using ACI. The disadvantages of ACI include loss of phenotype in monolayer culture and leakage of cell suspension when injecting it into focal lesions that had been covered by periosteum during the repair procedures. Uneven distribution of cells is another shortcoming.<sup>10</sup>

Allogeneous osteochondral transplantation has been used, especially for sports injury,<sup>6</sup> and 60%–88% good/excellent results have been reported at 4.5–7.8 years of follow-up.<sup>11,12</sup> Transmission of diseases and immune rejection<sup>13</sup> are pitfalls of the technique. Autogenous osteochondral transplantation was used by Hangody et al.<sup>7</sup> to treat focal osteochondral defects. Among 57 patients, 91% good to excellent results were noted after 3 years of follow-up. The disadvantage of this technique is poor integration of cartilage between the recipient site and chondral graft and hypertrophy of the chondral graft, which resulted in an irregular joint surface.

Based on these previous disadvantages, tissue engineering of cartilage emerged, and biotechnology of stem cells and scaffolds has progressed rapidly in recent years.<sup>14</sup>

### **Development of an ideal scaffold**

We have used gelatin, hyaluronic acid, and chondroitin-6-sulfate, a tripolymer, to mimic the matrix of cartilage. Gelatin had been used for hemostasis during surgery. Hyaluronic acid is a nonsulfated glycoaminoglycan (GAG) in the matrix of cartilage. Chondroitin-6-sulfate appears to promote more diffuse chondrogenesis

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and matrix production when combined with collagen II than when combined with collagen I.<sup>15</sup> The tricopolymer has a uniform pore size of about 180  $\mu\text{m}$  and adequate porosity of 75%.<sup>16</sup>

When porcine chondrocytes were seeded into the tricopolymer for 4 weeks, newly secreted matrix with lacunar formatting around chondrocytes could be seen with Alcian blue staining. Based on the technology of stem cell and scaffolding, allogeneous chondrocytes were cultivated in the tricopolymer to repair osteochondral defects in minipigs. Several findings are worth mentioning<sup>17</sup>: (1) Spontaneous healing as the defect filled with scaffolding alone did not result in good repair. (2) The subchondral bone plate was not restored in osteochondral defects by engineered cartilaginous tissue. (3) Full-thickness cartilage defects in an articular surface could be repaired by engineered cartilaginous tissue using the tricopolymer as a scaffold.

Because the tricopolymer mentioned above has not been approved for use in the human body, we have used atelocollagen, which has been used in humans by Kusaka et al. since 1987.<sup>18–21</sup> Ochi and colleagues cultivated autologous chondrocytes in thin scaffolds for 21–26 days before transplantation onto the articular surface of the knee to repair defects caused by trauma and osteochondritis dissecans, among other etiologies. At a follow-up of more than 25 months, the gross appearance was excellent. The hardness of the implanted site was similar to that of the surrounding cartilage.<sup>22</sup>

Wakitani and colleagues were the first to transplant human autologous stem cells into defects in osteoarthritic knees.<sup>23</sup> At 40 weeks after transplantation, the defects were covered with white soft tissue in which metachromasia was observed in almost all of the sampled tissue, and there was some hyaline cartilage-like tissue as well. Since 2005, we have been cultivating mesenchymal stem cells (MSCs) in atelocollagen with or without transforming growth factor beta (TGF $\beta$ ) for repairing full-thickness cartilage defects. The MSCs were size-sieved from bone marrow<sup>24</sup> and proved to be multipotent.<sup>25</sup> Preliminary results (reported at the 2007 Congress of Tissue Engineering and Regenerative Medicine — International Society) showed that the cartilaginous tissue induced from MSC by TGF $\beta$  could certainly repair full-thickness cartilage defects, as did the pure MSCs, although subchondral bone could not be restored by the induced MSCs.<sup>26</sup>

## Investigations in humans

Before tissue-engineered bioproducts are implanted into the human body, two basic conditions should be fulfilled: First, all bioproducts should be manufactured in good manufacturing practice (GMP) laboratories.

Second, all reagents used for production should be hand-inspected by health authorities. Finally, a well-designed protocol is necessary. Jakobsen et al.<sup>27</sup> suggested six guidelines.

1. Studies should be prospective with a clearly defined hypothesis and have a clearly defined primary endpoint; and they should be randomized controlled trials. Secondary endpoints are used only as supportive evidence.
2. Patient inclusion and exclusion criteria should be clearly established and reported. The recruitment rate should be reported.
3. The outcome measure should be validated for use in patients with cartilage injuries.
4. Outcome should be assessed by an independent investigator.
5. The timing of the outcome assessment should be clearly stated.
6. Detailed rehabilitation protocols should be established and reported.

We are fortunate to have a good tissue practice (GTP) cell manufacturing facility at the Biomedical Engineering Research Laboratories in the Industrial Technology Research Institute, Shinchu, Taiwan. At the time the paper was proofed, three patients with osteonecrosis of the medial femoral condyle had been treated by us with cartilage tissue induced from autologous MSCs. We expect that the results will be as good as those of mosaicplasty without its disadvantages, such as having a gap between the recipient site and the implant.

According to the slogan of the Orthopaedic Research Society of the United States, “Tomorrow’s treatments begin with today’s research.” Thus, the research on tissue engineering of cartilage will continue to progress.

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